make them inevitable or impossible, and steric factors may either accentuate or mask this effect. Specifically, it has been shown that the direct conversion of an *o*-hydroxybenzophenone oxime, whose configuration is such that the oximino hydroxyl group is *anti* to the phenolic hydroxyl group, to a benzisoxazole can be effected.

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[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY AND PHYSICS OF THE PENNSYLVANIA STATE COLLEGE AND THE PARKE, DAVIS & COMPANY RESEARCH LABORATORIES]

Sterols. XXVIII. Pregnanetriols from Pregnancy Urine

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In the course of our work on the isolation of various sterol derivatives from pregnant mares' urine we have isolated two triols possessing the empirical formula $C_{21}H_{36}O_3$. In some respects they appear to bear the same relationship to each other as do *allo*-pregnanediol and pregnanediol. Provisionally we are referring to them as pregnanetriol-A and pregnanetriol-B. Compound A is less soluble than the isomer in ethyl alcohol and so separates first in the isolation procedure. Both compounds melt in the neighborhood of 300° with slight decomposition. Compound A forms a triacetate melting at 136° whereas Compound B forms a triacetate melting at 168°. It was of interest to find that in the form of their acetates the solubility relationship is reversed and that the acetate of Compound B is less soluble in methyl and ethyl alcohols than is Compound A.

A search of the literature shows that Haslewood, Marrian and Smith,[1] in an investigation of the neutral carbinols of mares' pregnancy urine, obtained a saturated alcohol which they suggested might be a pregnanetriol. From the physical properties above presented it appears that B may be identical with the compound already described by Haslewood, Marrian and Smith.

The neutral fraction of mares' pregnancy urine, consisting mainly of esters of carbinols and hydrocarbons, was hydrolyzed with an excess of alkali and extracted with a large volume of benzene. After removal of the solvent the tarry residue was allowed to stand for a prolonged period in order to solidify and crystallize. The tar was removed by washing with an equal volume of ether and the crystalline residue fractionated by recrystallization from ethyl alcohol. The more insoluble fraction (pregnanetriol-A) was obtained in about twice the quantity of the more soluble fraction. A more satisfactory and almost quantitative separation can be made by acetylating the crude mixture and separating the two isomeric triacetates by means of solvents. The triacetate of pregnanetriol-A is quite soluble in methanol and can be crystallized only upon dilution with water whereas the triacetate of pregnanetriol-B is quite insoluble in methanol. These acetates give depressions in melting points when mixed with each other, and upon hydrolysis give the original triols with much sharper melting points.

Both triols give an orange red color with a green fluorescence when warmed with concentrated sulfuric acid, which is also characteristic of the pregnanediols. Neither isomer precipitates with alcoholic digitonin solution.

That these compounds have an angular methyl group between rings A and B is indicated by the fact that when heated with platinum black under the same conditions under which neoergosterol is transformed into dehydroneoergosterol[2] no naphthalene derivative is obtained.

Compound A contains an —OH group in the 20-position as is shown by the fact that it gives a positive iodoform reaction showing the presence of a CH_3CHOH— group. One of the other hydroxyl groups is probably at the usual 3-position, and since the third hydroxyl also can be acetylated, one can assume that it is not a tertiary hydroxyl. From the reactivity of the third hydroxyl group, about which we expect to publish later, we have indications that it is in the same position in the nucleus as the unreactive nuclear-OH group in the cortical hormone derivative of Reichstein.[3] Thus we may speculate on the possibility of this new compound being derived from the hormone of the adrenal cortex. Papers dealing with the structures

(1) Haslewood, Marrian and Smith, *Biochem. J.*, **28**, 1316 (1934).

(2) Honigmann, *Ann.*, **511**, 292 (1934).

(3) Reichstein, *Helv. Chim. Acta*, **19**, 29, 223, 402, 979, 1107 (1936).

of both compounds will be submitted soon for publication in THIS JOURNAL.

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Experimental

Extraction of Mixture of Triols.—An extract of the nonphenolic material of mares' pregnancy urine was hydrolyzed using a large excess of sodium hydroxide and steam distilling the volatile material during a period of three hours. The hydrolyzed product was extracted with benzene, the solvent evaporated, and the tarry residue allowed to stand until solidification and partial crystallization had taken place. One volume of ether was added and the crystalline product was filtered off. The crystals were washed with a small amount of cold benzene and then treated with Norite in alcohol. Upon concentration of the alcoholic solution to the point of crystallization a product separated which upon recrystallization gave pregnanetriol-A melting at 295–300° in a yield of about 10 mg. per gallon of urine. From the mother liquors a product was obtained which melts at about the same temperature as pregnanetriol-A, but gives a depression in melting point when mixed with it. The yield of Compound B was 6 mg. per gallon of urine. These products were purified by crystallization of their acetates followed by hydrolysis.

Acetate of Pregnanetriol-A.—A solution of 500 mg. of pregnanetriol-A in 20 cc. of acetic anhydride was refluxed for thirty minutes. The excess acetic anhydride was evaporated and the product crystallized from dilute methanol, since it was found to be very soluble in absolute methanol. The recrystallized product melted at 136°, uncorr.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.4; H, 9.3.

Acetate of Pregnanetriol-B.—A solution of 500 mg. of pregnanetriol-B in 20 cc. of acetic anhydride was refluxed for thirty minutes. The excess acetic anhydride was evaporated and the product crystallized from absolute methanol, in which it is quite insoluble. The melting point (168° uncorr.) corresponds to the acetate of the compound which was isolated by Marrian. When mixed with the acetate of pregnanetriol-A a depression in melting point was observed.

Anal. Calcd. for $C_{27}H_{42}O_6$: C, 70.1; H, 9.2. Found: C, 70.0; H, 9.3.

When a mixture of pregnanetriol-A and pregnanetriol-B was acetylated and crystallized from methanol, the triacetate of pregnanetriol-B was obtained in pure form upon two crystallizations from methanol, whereas the triacetate of pregnanetriol-A was obtained by adding 30% water to the first filtrate and cooling. Upon recrystallization pure pregnanetriol-A triacetate was obtained. This gives a much better way of separating the two isomers than crystallization of the free triols.

Pregnanetriol-A.—To a solution of 100 mg. of the triacetate of pregnanetriol-A, dissolved in 50 cc. of alcohol, was added an excess of potassium hydroxide. The product was refluxed for thirty minutes, water was added and the solid filtered. Upon crystallization from ethyl alcohol it melted at 295–300°.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.9; H, 10.8. Found: C, 74.6; H, 11.0.

Pregnanetriol-B.—A solution of 100 mg. of the triacetate of pregnanetriol-B was hydrolyzed in the same manner as pregnanetriol-A. It gave a product which upon crystallization from ethanol melted at 300–302°. It gave a depression in melting point of 25° when mixed with pregnanetriol-A. Its rotation in pyridine solution was found to be $[\alpha]^{25}D$ −41°.

Anal. Calcd. for $C_{21}H_{36}O_3$: C, 74.9; H, 10.8. Found: C, 74.8; H, 10.7.

Treatment of Pregnanetriol-A and -B with Platinum Black.—A mixture of both isomers of pregnanetriol was heated to 300° with platinum black catalyst under the same conditions under which neoergosterol is converted into dehydroneoergosterol. The product thus obtained when treated with picric acid solution gave no picrate, thus showing that no naphthol was formed. This is evidence of the presence of an angular methyl group between rings A and B.

Summary

Two triols have been isolated from the nonphenolic sterol fraction of mares' pregnancy urine. One of these compounds, pregnanetriol-A, is a new compound whereas the other, pregnanetriol-B, agrees in properties with the triol reported by Haslewood, Marrian and Smith. A speculation on the position of the –OH group in Compound A is presented, based upon experiments which are now in progress and will be reported later.

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